

Hepatotoxic Potentials of Food Additives on Liver Enzymes and Tissues of Wistar Rats

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Abstract

Background

Food additives such as monosodium glutamate (MSG), sodium benzoate (SB), and Sunset Yellow (SY) are widely consumed and have been linked to adverse hepatic effects. However, the available evidence is largely derived from in vitro or single-additive studies, with limited comparative in vivo data integrating biochemical and histopathological outcomes under uniform experimental conditions.

Objective

This study compared the hepatotoxic effects of MSG, SB, and SY in a Wistar rat model to address gaps in controlled in vivo evidence on multiple food additives.

Methods

Sixteen healthy Wistar rats were randomly assigned to four groups. The control group received a standard diet, while Groups II–IV received MSG, SB, and SY respectively. Hepatotoxicity was assessed by measuring serum activities of aspartate aminotransferase, alanine aminotransferase, and alkaline phosphatase, together with histopathological examination of liver tissue.

Results

The MSG and SB significantly increased aspartate aminotransferase (AST) and alkaline phosphatase (ALP) levels compared with controls ($p < 0.05$), indicating hepatocellular and cholestatic injury. Sunset Yellow did not significantly alter ALT levels ($p = 0.074$) but showed mild histopathological changes.

Conclusion

Both MSG and SB exhibited greater hepatotoxic effects than SY in Wistar rats. These findings highlight the need for routine safety re-evaluation of commonly consumed food additives, strengthened regulatory oversight, and further long-term studies to inform evidence-based public health policies.

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Introduction

Food dyes constitute a captivating category of food additives, as the colour of a product often influences its consumer appeal and can alter the taste, sweetness, and overall pleasantness.[1] Tartrazine, also referred to as E102, is an artificial dye widely utilised in developing nations as a saffron substitute.[2,3] It is frequently utilised to add colour to a variety of food and pharmaceutical items, such as soft drinks, cake mixes, and medicinal capsules.[2] Despite its advantages in food processing, exposure to tartrazine presents health risks and has been associated with a range of allergies in susceptible individuals.[4] Overexposure may lead to health issues, including neurobehavioral damage, psychopathological symptoms, itching, weakness, heat sensations, difficulty breathing, purplish skin patches, and sleep disturbances.[3] Furthermore, tartrazine has been linked to DNA-damaging effects in animal studies.[4]

Sunset Yellow (SY), also known as Orange Yellow E110, is a synthetic dye used in food colouring, notably found in orange sodas. This reddish-yellow dye is commonly used in the baking and biscuit industries.[5,6] To enhance flavour in food products, Monosodium glutamate (MSG) is used globally; it is a known version of the amino acid glutamate (AAG). It is employed in various food products, including meat, poultry, sauces, and soups.[7] Both SY and MSG have been linked to adverse effects such as allergic reactions, thyroid cancers, chromosomal damage, urticaria, hyperactivity, stomach pain, and, in the case of MSG, potential detrimental effects on growth and development, including brain damage, delayed skeletal development, behavioural abnormalities, and seizures.[8] Also, it has been linked to the Chinese restaurant syndrome and its symptoms. Furthermore, MSG has been observed to induce chromosomal abnormalities, sister chromatid swaps, and reduced replication and mitotic indices in human cells in a dose-dependent manner.[8]

Notably, sodium benzoate (SB) is a common preservative in soft drinks and beverages that inhibit microbial growth in acidic environments. It is also utilised in salad dressings, jams, fruit juices, and pharmaceuticals to maintain the freshness of liquid medications.[9]

The critical role of the liver's metabolic processes, which include the metabolism of foreign substances (xenobiotics), cannot be overemphasised. It serves as the primary site of contact for orally ingested xenobiotics, such as drugs, food additives, and environmental pollutants, thereby rendering it susceptible to injury from these compounds.[10,11] The liver primarily metabolises xenobiotic substances encountered by humans through food additives.[11] The by-products resulting from this metabolism can occasionally pose greater hazards than the original substances.[12] Potential biological impacts of xenobiotics include pharmacologic responses, toxicity, immunologic reactions, and cancer development.[13] Individual responses to xenobiotics vary not only with factors such as dose, individual heterogeneity, and genetic variables, but also with long-term exposure to low doses and environmental influences.[14] Xenobiotic-induced liver damage is a major contributor to acute liver failure, and the enzymes are routinely assessed to rate hepatic function.[12]

In Nigeria, cancer claims over 70,000 lives annually, with 5% of all cancer-related deaths attributed to liver cancer.[15] The causes of these liver cancers can be traced to environmental or genetic factors. To address food insecurity, there has been an increasing reliance on food additives such as MSG, SB, SY, and artificial sweeteners. Despite their beneficial applications, these additives also pose potential adverse health effects, particularly liver diseases and cholestasis.[16] Such conditions may progress to liver carcinoma, given that the liver is the primary organ responsible for metabolising xenobiotics.

Unfortunately, there is a limited body of research on the impact of these food additives on the liver in our local context. Previous studies [14] addressing this area have focused on xenobiotic substances encountered by humans through food additives, which are primarily metabolised by the liver.[13] The by-products resulting from this metabolism can occasionally pose greater hazards than the original substances.[14] Potential biological impacts of xenobiotics include pharmacologic responses, toxicity, immunologic reactions, and cancer development.[13] Individual responses to xenobiotics vary not only with factors such as dose, individual heterogeneity, and genetic variables, but also with long-term exposure to low doses and environmental influences.[15] This research endeavours to bridge this knowledge gap and shed light on the potential effects of additives on liver health. The data and perspectives obtained from this research will be essential for advancing understanding of the known adverse health effects of these chemicals and for developing effective strategies and interventions to mitigate these impacts.

In our current environment, there is a noticeable gap in research concerning the hepatotoxic effects of these food additives. Examining the influence of food additives on liver cells and enzymes is imperative, particularly in developing nations where the utilisation of additives in households, industries, and various products is less regulated or implemented inconsistently. Although several studies have demonstrated the hepatotoxic potential of SB, important knowledge gaps remain. Previous investigations have primarily focused on isolated aspects of SB toxicity, including genotoxicity in human hepatoma G2 (HepG2) cells, as assessed using the comet assay,[43] oxidative stress and inflammatory cytokines in experimental animals,[44] and its ability to aggravate pre-existing lipopolysaccharide-induced liver injury.[45] These studies have significantly advanced understanding of SB toxicity but were not designed to compare the relative hepatotoxic effects of different commonly consumed food additives under

identical experimental conditions. Moreover, most evaluated SB as a single exposure rather than examining its hepatotoxic profile alongside other widely consumed additives.

The present study addresses this gap by directly comparing the hepatotoxic effects of MSG, SB, and SY using a uniform *in vivo* Wistar rat model with identical dosage, exposure duration, biochemical assessment of liver enzymes, and histopathological evaluation. This comparative approach provides a more comprehensive assessment of the relative hepatic risks associated with these food additives. It generates evidence that may better inform food safety evaluation and regulatory decision-making.

Methods

Experimental design and Wistar rats selection

This controlled *in vivo* experimental animal study was conducted between August and October 2022 and was designed to evaluate and compare the hepatotoxic effects of MSG, SB, and SY on liver enzyme activities and liver histopathology in Wistar rats. The experimental procedures commenced with the acclimatisation of the Wistar rats, which were housed in standard cages under standard laboratory conditions in the animal facility. Adult Wistar rats weighing between 120 and 180 g were included in this study. Animals were selected based on predefined inclusion criteria, including appropriate strain (Wistar rats), an acceptable body weight range, normal physical activity, and absence of observable clinical abnormalities during the acclimatisation period. Rats were excluded if they were of a different strain, and those that had body weight below the specified range, or exhibited any signs suggestive of ill health, including lowered activity, abnormal posture, visible injury, poor coat condition, signs of infection, abnormal behaviour, or significant deviation in body condition, were considered unhealthy and excluded.

The test substances comprised MSG, SB, and SY, which were obtained from commercially available food-grade sources.

Distilled water served as the control treatment. The required quantities of MSG, SB, and SY were calculated based on the intended dose of 100 mg/kg body weight and accurately weighed using an analytical balance. Each compound was separately dissolved in distilled water to prepare individual treatment solutions. The prepared solutions were mixed thoroughly to ensure complete dissolution and administered at 1 mL per 100 g body weight, ensuring a consistent administration volume across all experimental groups.

The animals were randomly allocated into four experimental groups. Group I served as the control group and received distilled water only (1 mL/100 g body weight). Group II received MSG at a dose of 100 mg/kg body weight/day. Group III received SB at a dose of 100 mg/kg body weight/day, while Group IV received SY at a dose of 100 mg/kg body weight/day. All treatments were administered orally according to the experimental protocol.

Treatment and observations of the rats

In all groups, each rat received 1 mL/100 g body weight for 30 days. The rats were monitored and examined each day to assess their weight and responses to external stimuli. A similar research methodology had been used in a study by Ewenighi and colleagues.[19]

Dose calculation

$$V(ml) = \frac{W(kg) \times SD(mg/kg)}{CA(mg/mL)},$$

where V is the volume administered in mL; W is the weight of the rat in kg; SD stands for the standard dose in (mg/kg), and CA stands for the Concentration of the additive in (mg/mL).

After 30 days, the rats in each group were anaesthetised using chloroform vapour in a confined, transparent plastic container, following a method widely recommended in the literature.[20]

Blood samples were then collected via cardiac puncture into plain containers after disinfecting the surface with 70% alcohol. To collect serum from whole-blood samples, the samples were allowed to stand at room temperature for 1 hour. This allowed the collected blood samples to clot, with subsequent serum retraction. The serum was then separated for liver enzyme tests, including aspartate transaminase (AST), Alanine transaminase (ALT), and alkaline phosphatase (ALP). [18,21] Thereafter, the animals were dissected to extract the liver. The liver was preserved in 10% buffered formal saline for histopathological examination.[19] All reagents and solvents used were of analytical grade, maintaining the requisite precision and accuracy throughout the experimental procedures.

Serum liver enzyme analysis via colourimetric method

Liver enzymes were measured using a standard method.[20] Serum ALP catalyses the hydrolysis of phenolphthalein monophosphate to phosphoric acid and phenolphthalein; in an alkaline medium, the released phenolphthalein develops a pink colour that is measured spectrophotometrically at 405 nm.[46] Before testing, reagents and samples were brought to room temperature. A mixture of 3000 µL ALP reagent and 50 µL sample was homogenised, and absorbance was read at 1, 2, and 3 minutes. The ALP activity was calculated using the formula $U/L = 3300 \times \Delta A / \min$. [16] Measurements were performed using a spectrophotometer (Erba Chem 7) at 510 nm.[21]

Furthermore, ALT was assessed by its role in transferring an amino group from alanine to oxoglutarate, producing pyruvate, which reacts with 2,4-dinitrophenylhydrazine (DNPH) to form a red-brown compound measurable at 546 nm. Absorbance was read after 5 minutes.[17] Similarly, AST activity was evaluated by measuring oxaloacetate hydrazone formed with 2,4-dinitrophenylhydrazine. Samples and reagents were equilibrated, prepared

according to the manufacturer's instructions, and read at 546 nm after 5 minutes.[18]

Histopathological techniques

The fixed liver specimens underwent a gross examination in triplicate and were then processed using the Leica TP1020 Tissue Processor (Leica Biosystems, Germany). [31, 22] This automated system facilitates fixation, dehydration, and infiltration processes involved in preparing histological tissue samples. The tissue embedding phase was performed using the HistoCore Arcadia modular tissue embedding system (Leica Biosystems, Germany). This system includes two essential components: the Arcadia H heated embedding workstation and the Arcadia cold plate (-14°C), which provides rapid cooling and solidification of paraffin blocks and operates at approximately -10°C to -20°C . This configuration provides flexibility in organising the embedding workflow to accommodate specific laboratory requirements and workspace limitations. The operational principles of these automated machines are consistent with the foundational concepts discussed in routine manual and rapid processing.[20] In addition, tissues were labelled with unique IDs, sectioned at a thickness of $4\text{ }\mu\text{m}$ using the HistoCore MULTICUT R Semi-Automated Microtome with a horizontal blade (Leica Biosystems, Germany). Routine (H&E) and special stains, including Periodic acid-Schiff and Masson's trichrome, assessed tissue architecture, glycogen storage, abnormalities in carbohydrate metabolism, fibrosis, and connective tissue integrity, thereby aiding in the evaluation of liver damage and fibrosis.

Haematoxylin and eosin staining techniques

Notably, the haematoxylin and eosin staining method uses hematoxylin. This basic dye stains nucleic acid-rich basophilic structures blue-purple, and eosin, an acidic counterstain that imparts pink to red hues to RBCs, cytoplasm, and collagen.[19,21] Tissue sections were warmed at 40°C , deparaffinized in xylene, hydrated in graded alcohol, and stained with Mayer's

hematoxylin (15 min) and eosin (2 min). Furthermore, $4\text{ }\mu\text{m}$ tissue sections were then dehydrated, cleared in xylene, mounted in distrene-plasticiser-xylene (DPX), and examined.[20,49]

Periodic acid schiff's (PAS) technique

Importantly, this test demonstrated the presence of glycogen, inflammation, and hepatic injury by oxidising 1:2 glycol groups to dialdehydes, which reacted with Schiff's reagent to form a magenta compound.[20] The $4\text{ }\mu\text{m}$ tissue sections were deparaffinized in xylene, hydrated in alcohol, treated with Periodic Acid (5 min), rinsed, and stained with Schiff's solution (20 min).[17] Counterstaining with hematoxylin followed, and sections were blued, dehydrated, cleared, and mounted for examination.[17, 49]

Trichrome staining technique

This technique relies on electrostatic binding of dyes to tissue, enabling selective sequential staining. Initially, RBCs are stained with Orange G, which binds more strongly to them than collagen or muscle. Acid fuchsin, with larger particles, stains muscle and collagen. Differentiation with phosphotungstic acid removes acid fuchsin from collagen, followed by aniline blue or light green for collagen staining.[18] Additionally, the $4\text{ }\mu\text{m}$ tissue sections were deparaffinized, treated with various dyes, and mounted for examination.[18, 49]

Haematoxylin van gieson (HVG) staining

This method relies on electrostatic attachment of dyes to tissue, enabling selective sequential staining with anionic dyes. Initially, Orange G, a small particle dye, stains RBCs more strongly than collagen or muscle. Acid fuchsin, with larger particles, stains collagen and muscle, and differentiation with phosphotungstic acid removes acid fuchsin from collagen. Finally, aniline blue or light green stains collagen fibres.[20,17] Also, the $4\text{ }\mu\text{m}$ tissue sections are deparaffinized in xylene, stained, rinsed, counterstained with neutral red, and mounted in DPX for examination.[21,18,49]

Statistical analysis

Statistical analyses were performed using IBM SPSS Statistics version 23 (IBM Corp., Armonk, NY, USA). All continuous variables are presented as mean $\pm$ standard deviation (SD). Weekly body weight data were analysed by comparing each treatment group (SY, SB, and MSG) individually with the control group at each weekly time point using independent samples t-tests. Similarly, serum liver enzyme activities (AST, ALT, and ALP) in each treatment group were compared with those of the control group using independent-samples t-tests.

To evaluate differences among the three-food additive-treated groups (SY, SB, and MSG), one-way analysis of variance (ANOVA) was performed separately for AST, ALT, and ALP activities. The ANOVA results are presented as F-statistics with corresponding degrees of freedom [F (2,8)] and p-values. All statistical tests were two-tailed, and a p-value < 0.05 was considered statistically significant.

Ethical considerations

All experimental procedures involving animals were approved by the Sub-Ethics Committee of the Faculty of Health Sciences and Technology, Ebonyi State University, Abakaliki, Nigeria (Approval No.: FHST/SEC/2023/016), prior to study commencement. Animal use and care were conducted in accordance with the Guide for the Care and Use of Laboratory Animals, 8th edition. They were consistent with the principles of Replacement, Reduction, and Refinement (3Rs) embedded in current international guidance for animal research. [47] Experimental procedures were further aligned with Directive 2010/63/EU, which requires the application of the 3Rs in all aspects of animal care and use, as well as with contemporary ethical review frameworks that emphasise harm-benefit assessment and the minimisation of animal pain, suffering, and distress.[48]

Results

Effects on body weight and liver enzyme activities

As shown in Table 1, data on weekly weights of Wistar rats treated with SY, SB, MSG, and Control group over 30 days are presented as mean $\pm$ standard deviation (SD). The control group exhibited a steady increase in mean body weight over the 30 days, rising from 160 ± 14 g in week 1 to 168 ± 17 g in week 4. The SY-treated rats also gained weight during the study period. However, their mean body weights remained consistently lower than those of the control group, with statistically significant differences observed at all weekly time points. A similar pattern was observed in the MSG group, with body weight increasing progressively from week 1 to week 4 but remaining significantly lower than the corresponding control values throughout the experiment. In contrast, the SB-treated group showed slightly lower mean body weights than the control group at each weekly interval, but these differences were not statistically significant. Overall, Table 1 indicates that SY and MSG were associated with reduced weight gain compared with the control group. In contrast, SB did not significantly affect body weight over the exposure period.

Data comparison of liver enzyme activities in Wistar rats exposed to SY, SB, MSG, and control group are presented as mean $\pm$ SD as shown in Table 2. The liver enzyme results showed a clear biochemical effect of additive exposure. Serum AST activity was significantly higher in all treatment groups than in controls, increasing from 24.00 ± 8.03 U/mL in the control group to 40.15 ± 1.89 U/mL in the SY group, 43.37 ± 4.31 U/mL in the SB group, and 37.61 ± 5.07 U/mL in the MSG group. The ALT activity followed a more selective pattern. Although ALT was numerically higher in the SY group than in the control group, the difference was not statistically significant ($p = 0.074$). By contrast, significant elevations were observed in the SB group (43.57 ± 3.95 U/mL, $p = 0.005$) and especially in the MSG group, which recorded the highest ALT value of 103.46 ± 4.20 U/mL ($p < 0.001$). ALP activity showed the strongest overall response, rising from 48.80 ± 1.70 IU/L

in controls to 102.01 ± 4.05 IU/L in the SY group, 103.46 ± 4.20 IU/L in the SB group, and 79.14 ± 6.63 IU/L in the MSG group, with all increases reaching statistical significance.

Table 1. weekly mean weights ($\pm$ sd) of Wistar rats treated with sunset yellow, sodium benzoate, monosodium glutamate, and control group over 30 days

Week	Sunset Yellow		Sodium Benzoate		Monosodium Glutamate		p-value	t-value (MSG vs Control)	p-value
	Control (Mean $\pm$ SD)	(Mean $\pm$ SD)	t-value (SY vs Control)	p-value	(Mean $\pm$ SD)	t-value (SB vs Control)			
Week 1	160 $\pm$ 14	150 $\pm$ 15	2.134	0.034*	155 $\pm$ 12	1.567	0.112	1.934	0.043*
Week 2	162 $\pm$ 15	152 $\pm$ 17	2.221	0.029*	158 $\pm$ 13	1.765	0.091	1.843	0.042*
Week 3	165 $\pm$ 16	155 $\pm$ 18	2.465	0.022*	160 $\pm$ 14	1.893	0.087	1.756	0.047*
Week 4	168 $\pm$ 17	158 $\pm$ 19	2.673	0.018*	162 $\pm$ 15	1.923	0.085	1.895	0.043*

Note: This table shows the weekly mean weights and standard deviation (mean $\pm$ SD) of Wistar rats treated with Sunset Yellow, Sodium Benzoate, Monosodium Glutamate, and Control group over 30 days. The weights were recorded every day for each group. The t-values and p-values represent statistical comparisons of the treatment groups with the control group for each week ($p < 0.05$ is significant). Mean $\pm$ standard deviation (SD).

Table 2. Comparison of liver enzyme activities in Wistar rats exposed to sunset yellow, sodium benzoate, monosodium glutamate, and control group

Variable	Control (Mean ± SD)	Sunset Yellow (Mean ± SD)	Sodium Benzoate		Monosodium Glutamate		p-value	t-value (MSG vs Control)	p-value	t-value (MSG vs Control)
			t-value (SY vs Control)	p-value	t-value (SB vs Control)	p-value				
AST (U/mL)	24.00 ± 8.03	40.15 ± 1.89	3.341	0.024*	43.37 ± 4.31	4.253	37.61 ± 5.07	2.867	0.010*	0.035*
ALT (U/mL)	32.61 ± 3.04	43.31 ± 6.04	3.119	0.074	43.57 ± 3.95	4.393	103.46 ± 4.20	27.337	0.005*	<0.001*
ALP (IU/L)	48.80 ± 1.70	102.01 ± 4.05	24.161	<0.001*	103.46 ± 4.20	24.140	79.14 ± 6.63	8.861	<0.001*	0.002*

Note: Table 2 presents the liver enzyme activities (AST, ALT, and ALP) of Wistar rats exposed to Sunset Yellow, Sodium Benzoate, and Monosodium Glutamate, compared with the control group. Significant increases in enzyme activities were observed in the experimental groups (Sunset Yellow, Sodium Benzoate, and Monosodium Glutamate) compared with the control, with varying levels of statistical significance. The t-values and p-values are shown for each comparison ($p < 0.05$ is significant, $p > 0.05$ is not significant). Abbreviation key: AST = Aspartate Aminotransferases; ALT = Alanine aminotransferases; ALP = Alkaline phosphatase. Mean $\pm$ standard deviation (SD).

Comparative effect of additives on serum activity of AST, ALT, and ALP

Assessment of the effects of additives on serum activity is presented in Table 3. Data are shown as mean $\pm$ SD. The group of rats that were fed with SB exhibited the highest AST activity, while the lowest AST activity was observed in the group fed with MSG.

For ALT activity, the group fed with MSG had the highest level, while the lowest was recorded in the group fed with SY. Regarding ALP activity, the group fed with SB showed the highest level, while the lowest was observed in the group fed with MSG.

Table 3. Comparative analysis of the effect of food additives on AST, ALT and AST

Variables	Sunset Yellow	Sodium benzoate	Monosodium glutamate	F-value	p-value
	Mean $\pm$ SD	Mean $\pm$ SD	Mean $\pm$ SD		
AST (U/mL)	40.15 $\pm$ 1.89	43.37 $\pm$ 4.31	37.61 $\pm$ 5.07	1.904	0.211
ALT(U/mL)	43.31 $\pm$ 6.04	43.57 $\pm$ 3.95	103.45 $\pm$ 4.20	212.145	0.001
ALP (IU/L)	102.01 $\pm$ 4.05	103.46 $\pm$ 4.20	79.14 $\pm$ 6.63	26.343	0.001

Note: AST (U/mL): No statistically significant difference in AST levels between the effects of Sunset Yellow, Sodium Benzoate, and Monosodium Glutamate ($F = 1.904$, $p = 0.211$). ALT (U/mL): A statistically significant difference in ALT levels was observed, with Monosodium Glutamate showing a much higher effect compared with Sunset Yellow and Sodium Benzoate ($F = 212.145$, $p = 0.001$). ALP (IU/L): A statistically significant difference in ALP levels was found, with Monosodium Glutamate showing a significantly lower effect compared with Sunset Yellow and Sodium Benzoate ($F = 26.343$, $p = 0.001$).

Abbreviation key: AST, Aspartate Aminotransferases; ALT, Alanine aminotransferases; ALP, Alkaline phosphatase. Mean $\pm$ standard deviation (SD).

Histopathology reports

Histopathological results are presented in Figure 1 (Plates 4.1 to 4.4) and Figure 2 (Plates 4.5 to 4.8). All plates show X200 magnification.

In Figure 1, Plate 4.1 shows a Haematoxylin-Eosin-stained liver section of a Group 1 normal control Wistar rat that was given standard feed and water without intervention.

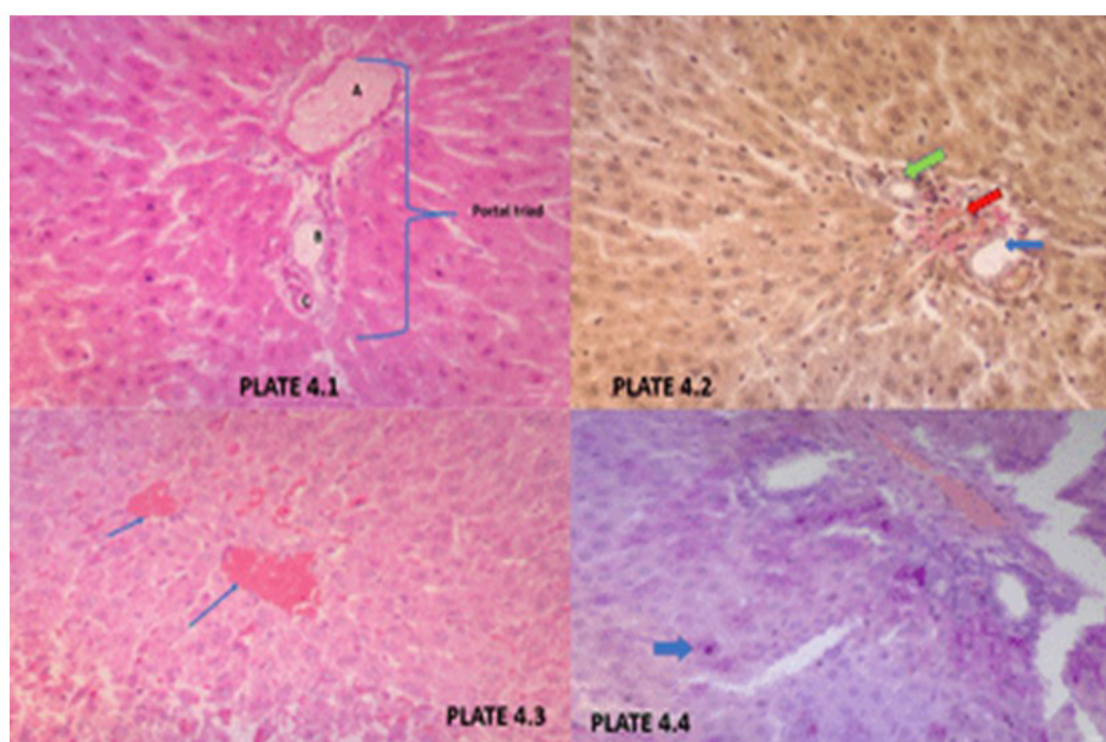


Figure 1. Photomicrograph of livers of Group 1 rats stained with Haematoxylin-Eosin (Plate 4.1) or Haematoxylin Van Gieson (Plate 4.2); Group 2 rats stained with Haematoxylin-Eosin (Plate 4.3) or Periodic Acid Schiff (Plate 4.4), Mag. X200

Letter A indicates the portal vein, B the Artery, and C the Bile duct, also called the portal triad. The clear sinusoidal spaces and hepatic plates appear normal with Haematoxylin and Eosin staining suggestive of normal liver tissue. Plate 4.2 illustrates HVG -stained liver section of Wistar rat of group 1, normal control, that was given standard feed and water without intervention. The section shows portal vein (Blue arrow), Artery (red arrow) and bile duct (Green arrow) also called portal triad. The clear sinusoidal space and hepatic plates appear normal, reminiscent of the normal liver with HVG-staining. Plate 4.3 depicts a group 2 Wistar rat liver section stained with Haematoxylin and Eosin. The rats were given standard feed, water and intervened with monosodium glutamate. The section shows moderate hepatic damage (blue arrows) and inflammatory background indicative of hepatic injury. Plate 4.4 shows a Wistar rat liver section stained with PAS. The rat was given standard feed, water and intervention with monosodium glutamate. The section shows apoptotic figures (blue arrows) also known as the councilman body which is a remarkable indicator of hepatic injury (Figure1).

In Figure 2, Plate 4.5 depicts an HVG stained liver section of group 3 Wistar rat given standard feed, water and intervened with sodium benzoate. It shows apoptotic figures, a thumbnail cellular appearance and remarkably a damaged liver (blue arrows) consistent with severe hepatic injury associated with drugs or toxins. Plate 4.6 illustrates a group of 3 Wistar rat liver sections stained with Haematoxylin and Eosin. The rats were given standard food and water and administered sodium benzoate. The section reveals a marked inflammatory reaction, a councilman body (blue arrow) and general damage to the hepatic architecture. Plate 4.7 displays a group 4 Wistar rat HVG-stained liver section. The rat was given standard feed, water and intervened with SY. The section reveals moderate hepatic injury and response of Kupffer cells (red arrow) within the lining of the sinusoids with staining. Plate 4.8 shows a Mason trichrome stained liver section of group 4 Wistar rat fed with standard feed and water and treated with sunset yellow. Section reveals a moderate hepatic injury and the response of Kupffer cells (yellow arrow) within the lining of the sinusoids.

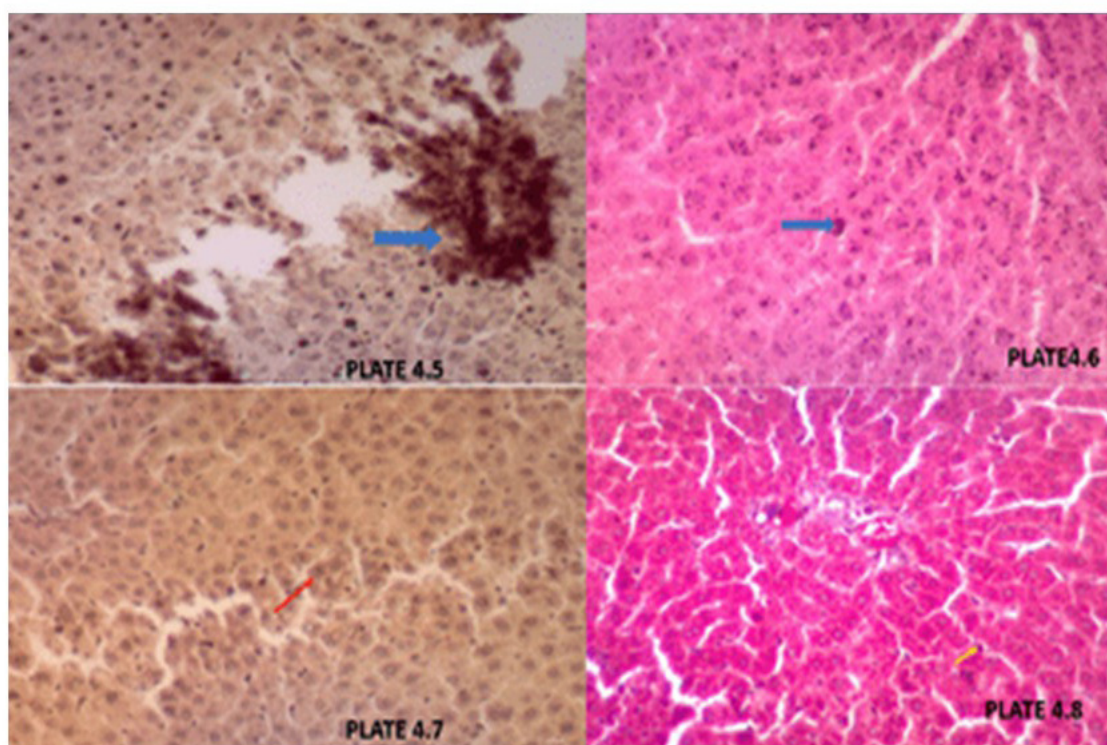


Figure 2: Photomicrograph of livers of Group 3 rats stained with Haematoxylin Van Gieson (Plate 4.5) or Haematoxylin-Eosin (Plate 4.6); Group 4 rats stained with Haematoxylin Van Gieson (Plate 4.7) or Mason trichrome stain (Plate 4.8), Mag. X200

Discussion

The investigation sought to determine the impact of daily oral administration of three commonly used food additives, SY, SB, and MSG, to laboratory animals for 30 days on physiological profiles, liver enzyme levels, and liver tissue cells. The study's findings demonstrated significant changes in several key parameters. It was found that after administering SY orally for 1 month, AST and ALP activities increased significantly. Although the increase in ALT activity was not statistically significant compared with the control group, this finding aligns with previous research.[19]; that AST elicited a notable increase in the enzyme ($p \leq 0.05$).

Previous research has consistently shown that consuming food additives such as SB and MSG leads to a notable increase in serum AST, ALT, and ALP levels.[23] ALP, predominantly located in the canalicular and sinusoidal membranes of the liver, acts as a marker for liver damage. Elevated serum ALP levels are linked to liver dysfunction and cholestatic liver disease.[20] The elevated levels of hepatic serum enzymes, particularly AST, in rats suggest increased permeability and damage to hepatic cells.[30]

The ALT enzyme is a well-established marker of insulin resistance, diabetes mellitus, and obesity, all of which are significant risk factors for coronary heart disease. Additionally, it is a sensitive indicator of liver damage.[32,33] In our study, the lack of a significant impact of SY on serum ALT activity suggests its relative safety, indicating fewer adverse effects on liver function and tissues than other compounds. These findings underscore the importance of understanding the physiological effects of commonly used food additives and highlight the need for further investigation into their potential health implications. The administration of SB led to a significant elevation in serum AST, ALT, and ALP levels compared with the control group (see Table 2). This aligns with a previous study.[25] which reported significant increases in all

serum liver enzyme levels compared with baseline. Previous research has consistently demonstrated that food additives, including SB, significantly raise serum AST, ALT, and ALP activities. These findings were attributed to hepatocellular damage from SB toxicity, as evidenced by vacuolation, swelling, necrosis, and pyknosis in liver cells.[34] The increased serum AST and ALT levels in rats, consistent with prior research, were linked to liver dysfunction and hepatocellular impairment, leading to the release of higher-than-normal levels of intracellular enzymes into the bloodstream.[35] SB induced liver dysfunction, as evidenced by significant elevations in serum ALT, AST, and ALP levels.

In the bloodstream, SB binds to plasma proteins, facilitating its transport to various tissues. In the liver, it undergoes conjugation with glycine, yielding hippuric acid.[36] The observed increases in serum enzyme activities, including ALT, AST, and ALP, following SB exposure, mirror findings from studies involving rats treated with N-nitrosodimethylamine [37] or N-nitrosamines.[38] Alkaline phosphatase is widely distributed on cell surfaces in various human tissues, particularly in the intestine, liver, bones, spleen, and kidneys. Its specific localisation within sinusoidal and bile canalicular membranes could explain its elevation in serum levels observed in our study following SB administration. These results highlight the potential hepatotoxic effects of SB and emphasise the need for further investigation into its impact on liver function and overall health.

Similarly, administration of MSG resulted in a markedly significant increase in ALT and ALP activity (p -value= 0.001 and 0.002, respectively). There was no statistical difference in AST activity within the separate groups (see Table 3). These findings align with a similar study,[25] in which all liver enzymes showed highly significant differences compared with the control group. The propensity of MSG to readily dissociate, releasing free glutamate, could contribute to the observed effects.

The reduction in glutamate levels results in the production of ammonium ions (NH_4^+), which can be toxic unless metabolised in the liver via the urea cycle. The potential accumulation of ammonium ions due to increased glutamate levels from MSG consumption could lead to liver damage, subsequently resulting in the release of the ALT enzyme. Furthermore, the elevation in enzyme levels may be attributed to the generation of free radicals, which interact with polyunsaturated fatty acids in cell membranes, causing damage to mitochondrial and plasma membranes and leading to enzyme leakage.[39] Cellular damage triggers the release of enzymes such as ALT, AST, and ALP into the bloodstream, with their levels serving as indicators of the nature and extent of the inflicted damage. [40,50]

Notably, photomicrographs of liver sections further support the biochemical findings. The control Wistar rat exhibited a normal, clear sinusoidal space, indicative of healthy liver tissue, when stained with H&E and HVG. In contrast, the liver section from group 2 Wistar rats treated with MSG showed moderate hepatic damage and an inflammatory background, as evidenced by H&E staining. The same section revealed apoptotic figures, known as Councilman bodies, which are notable indicators of hepatic injury when stained with PAS.

Importantly, the liver section from group 3, which was treated with sodium benzoate, displayed apoptotic figures, a thumbnail cellular appearance, and a remarkably damaged liver when stained with the HVG method.[20] These observations are consistent with severe hepatic injury associated with drugs or induced additives.

Lastly, the liver section from group 4, which was treated with SY, showed relatively moderate hepatic injury and Kupffer cell responses within the sinusoidal lining when stained with HVG. The tissue section stained with Mason trichrome further highlighted moderate hepatic injury and the response of Kupffer cells

within the sinusoidal lining. These combined biochemical and histological findings underscore the potential hepatotoxic effects of MSG, SB, and SY, emphasising the need for further investigations into their impact on liver function and overall health.

Strengths and study limitations

This study possesses several notable strengths. First, it employed a controlled comparative experimental design that enabled the direct evaluation of three commonly consumed food additives (MSG, SB, and SY) under identical experimental conditions, thereby minimising inter-group variability. Second, the integration of biochemical liver enzyme assays (AST, ALT, and ALP) with complementary histopathological examinations using multiple staining techniques (Hematoxylin and Eosin, PAS, Masson's trichrome, and HVG) provided robust and complementary evidence of hepatotoxicity. Furthermore, the study utilised standardised dosing, treatment duration, animal selection criteria, and experimental procedures, enhancing the internal validity and reproducibility of the findings. Collectively, these methodological strengths provide a comprehensive comparative assessment of the relative hepatotoxic effects of these widely used food additives.

Meanwhile, limitations include the absence of complementary in vitro and human-based validation studies to confirm the observed effects and elucidate the underlying mechanisms. Furthermore, mechanistic biomarkers of oxidative stress, inflammation, apoptosis, or fibrosis were not investigated, restricting mechanistic interpretation of the observed biochemical and histopathological alterations.

Conclusion

The findings of this study demonstrate that the investigated food additives exerted varying effects on liver health, with MSG and SB producing greater evidence of hepatotoxicity than SY. SY showed no significant effect on serum ALT activity,

suggesting comparatively lower hepatotoxic potential under the conditions of this study. These findings underline the need for cautious assessment of the potential hepatotoxic effects of dietary additives, calling for future research to completely examine their impact on liver health. Future research on food additives should focus on establishing a clear timeline of exposure to determine the threshold at which adverse effects begin to manifest. This should involve defining exposure duration, correlating exposure with effects, and comparing observed effects across varying exposure durations to understand how toxicity progresses over time.

Author's contribution

UUA and ON provided supervision throughout the project, including overseeing the work and examining the photomicrographs. US, S IO, and UVU reviewed and contributed to the manuscript. PI and UBM handled tissue processing. VN, IAO, KME, and ODA conducted literature searches, performed data analysis, and contributed to the manuscript preparation. OVC was responsible for feeding the experimental animals throughout the duration of the experiment.

Conflict of interest declaration

The authors declare that they have no conflicts of interest regarding the publication of this manuscript.

Generative AI

The authors used a generative artificial intelligence (AI) tool solely to assist with language editing and improve the clarity and readability of the manuscript. All scientific content, data analysis, interpretation of results, and conclusions were developed and verified by the authors, who take full responsibility for the accuracy and integrity of the work.

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